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Chronic Disabling Dermatoses

ANNUAL PROGRESS REPORT

by

Albert M. Kligman, M. D.

February 1967
(For the period 1 March 1966 to 28 February 1967)

U. S. Army Medical Research and Development Command

Washington, D. C. 20315

Annual Report to the Commission on Cutaneous Diseases
of the Armed Forces Epidemiological Board

Contract No. DA-49-193-MD-2137

University of Pennsylvania

Philadelphia, Pennsylvania 19104

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Annual Progress Report

Chronic Disabling Dermatoses

Period covered by the report: 1 March 1966 to 28 February 1967

Responsible investigator(s) and name of institution:

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Annual report to the Commission on Cutaneous Diseases of the Armed Forces
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ABSTRACT

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   Annual Report to the Commission on Cutaneous Diseases of the Armed Forces Epidemiological Board

Summary

Our researches have stressed two areas of outstanding importance in military dermatology:

(1) cutaneous bacteriology and
(2) the pathogenesis of sweat retention syndromes

It is unequivocally established that bacterial skin infections, primary or secondary, are a major source of disability, especially in tropical theatres. Moreover, a significant proportion of new arrivals experience sweat retention with all this entails in impairment of heat regulation and miliarial eruptions.

7. Key words:

miliaria
occlusion
anhidrosis
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quantitative techniques
geometric mean
normal flora
antibacterial agent
Declomycin
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resident organisms

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List of Illustrations

Sweat Retention Studies

The studies fall into two main divisions:

(1) the role of the cutaneous microflora in establishing sweat retention and
(2) analysis of the pathogenetic mechanisms of anhidrosis.

1. Relationship of Microflorc to Sweat Retention Syndromes

Strongest support for the thesis that bacteria may incite miliaria is to be found in the classic studies of O'Brien.[1,2,3] By observing many sections in the early stages of miliaria rubra, numerous bacteria were often densely congregated in the sweat pore. Furthermore, by applying agar cups streaked with Staphylococcus aureus, O'Brien succeeded in reproducing miliaria rubra. In Texas, Lyons et al[4] argued for a bacterial etiology by showing that subjects who developed miliaria harbored many more bacteria on their skin; in addition, treatment with topical antibacterial agents greatly shortened the time for cure.

Among the experimental ways of producing miliaria is keeping the skin hydrated for a couple of days by means of wet dressings; this produces an anhidrosis which lasts for days, long after the skin has dried out. How this happens is unknown. We have shown that simply covering the skin with Saran Wrap for two days will produce a near complete anhidrosis in the majority of subjects, whereas solid anhidrosis in all subjects occurs after four to six days. Histologic study after four to six days reveals no keratotic plug or other abnormality which might explain the obstruction to sweating. It occurred to us to test whether bacterial growth might not be involved since we have established that the resident population of organisms increased by several orders of magnitude under Saran Wrap. We undertook therefore, tc prevent bacterial multiplicatic s by pretreatment of the forearm skin with a variety of antibacterial agents. A known amount was deposited on the skin by applying alcoholic or aqueous solutions and evaporating the solvent under air. After six days one portion of the occluded site was sampled for bacteria after which the subjects were sweated for 30 minutes in a chamber at 50% R.H. and 105°F. The subjects were preselected in that each regularly developed miliaria when sweated after six days of occlusion. Fig. 1 correlates the development of miliaria with the effectiveness of the agent in suppressing bacterial growth. It is evident that prevention of bacterial over-growth either eliminates miliaria or reduces it significantly.

In cases where a material antibacterial effect was not achieved, miliaria was provoked by sweating almost as readily as on the control site. Practically all of the agents were effective in a majority of the test subjects. It is not clear why bacterial repression was not attained in all subjects but this was true for some of the subjects with each test agent. In some cases, selection of resistant strains was evident.

We propose that this study is an adequate model for what happens when an unaccommodated subject enters a tropical area. Profuse sweating hydrates the horny layer and enables a great increase in the bacterial flora. By some unknown means bacterial overgrowth induces anhidrosis and in predisposed subjects the interference with sweat delivery results in ductal rupture and the clinical manifestations of miliaria. Although the nature of the obstruction has not been determined, we have established that it is superficially located in that stripping with Scotch Tape immediately relieves the anhidrosis induced by occlusion.[5] These results suggest the practicality of applying an antibacterial preparation topically in order to prevent the sweat retention syndrome. A suitable formulation would be non-greasy, non-odorous, non-toxic, non-colored and highly substantive

to skin. We are planning to investigate various antibacterial substances in different vehicles in order to meet these requirements.

Fig. 1
Prevention of Miliaria by Application of Anti-Bacterial Agents Prior to Occlusion with Saran Wrap

Agent	Proportion of Subjects with more than 90% Reduction in Bacterial Numbers in Comparison with Control	Proportion with Significant Reduction in Miliaria
Polymyxin B	4/6	4/6
Oxacillin	4/6	4/6
Hexachlorophene	3/6	2/6
Furacin	3/5	2/5
Bacitracin	2/5	4/5
Iodine	3/5	3/5
Tetracycline HCl	3/3	2/3
Neomycin	8/11	8/11
All Groups	30/47	29/47

II. Analysis of Mechanisms of Anhidrosis

Earlier experiments believed that poral closure by keratotic plugs adequately explained the failure to sweat in anhidrotic areas. Such changes are now recognized to be secondary to ductal rupture and not antecedent to it. More careful study of the anatomy of the sweat pore shows that what was previously regarded as a plug is indeed the normal horny substance which fills the 'beaker' through which the sweat duct spirals in the terminal portion of the horny layer. Previous students have tended to study the skin after the development of a clinical miliarial disturbance, neglecting the anhidrotic state which necessarily precedes and which is unaccompanied by any gross or microscopic pathology. Our particular concern has been the development of methods for experimentally investigating the nature of the anhidrotic state.[5] As a result of studies of anhidrosis associated with chronic dermatitis or produced experimentally we have characterized under the designation of 'high level blockade' a particular variety which has as a common denominator an obstruction in the intracorneal superficial portion of the eccrine duct. The criteria on which a diagnosis of high level blockade rests are as follows:

1. Stripping with Scotch Tape immediately reestablishes sweating.
2. The iontophoresis of methylene blue fails to produce a 'pore pattern'; the dye cannot traverse the duct.
3. Dilatation of the duct after thermal stimulation accompanied by formation of a PAS positive diastase negative cast in the terminal portion of the duct.

Our investigations show that high level blockade accounts for the great majority of the familiar types of clinical and experimental anhidrosis, viz: chronic dermatitis, thermal, mechanical and chemical injuries and occlusion. The antiperspirant action of aluminum salts, so extensively used in axillary hygiene, cannot be explained in this fashion. An analysis of sweat gland histochemistry and physiology of aluminum anhidrosis has led us to propose that greatly increased resorption of water by the dermal duct 'siphons' off the sweat before it reaches the surface.[7] Secretory activity of the gland does not cease nor is there any block to interfere with the passage of sweat. These observations confirm that the duct is not merely a passive conduit for the transport of sweat. Other anhidrosis producing chemicals are being studied.

Cutaneous Bacteriology

We have intensified our studies of the cutaneous microflora. In addition to two laboratories of the Duhring Laboratories, a new laboratory has been established in our prison facility. The large number of samples from human volunteers can be processed on the premises. We are pursuing six main lines of investigation.

I. Development of Quantitative Techniques for Determining the Density and Composition of the Cutaneous Microflora

To date, the most satisfactory procedure has been scrubbing in a buffered solution of Triton X-100 as described by Williamson and Kligman.[8] Though accurate, this method is troublesome, expensive and time consuming. We are experimenting with simpler techniques which may be adequate for certain types of studies. Touch plates have been found valuable when the population density is high. By correlating quantitative counting with touch plate figures, reasonably good semi-quantitative estimates can be made quickly and easily. Successful applications include studies of efficacy of topical antibacterial substances provided that the test agent can reduce the original flora by more than 90%. In addition, the method is suitable for agents which effectively prevent the logarithmic increase in bacterial numbers after occlusion with Saran Wrap. Highly active antibacterials can be discriminated from less effective ones.

A different method of statistical treatment of the bacterial counts in the population has been a major methodological advance in our quantitative studies of the normal flora. The distribution is decidedly not normal when arithmetic averages are used largely owing to the existence of individuals with extremely high counts. It develops however that a plot of the logarithm of the count rather than the count itself follows the normal Gaussian distribution curve. Axillary counts in well over two hundred samples illustrate the remarkably good agreement with the normal bell shaped curve. (Fig. 2) Thus, the geometric mean is a statistic which conveniently characterizes bacterial density in a given population for a specific region.

II. Normal Bacterial Flora of the Skin

We deem it essential to the understanding of the pathological states caused by bacteria to delineate the normal flora qualitatively and quantitatively, for all major skin areas. The problem of classifying the resident organisms has many more complexities than formerly supposed. Previous accounts have been oversimplified and some species identifications are not acceptable. We have so far collected information for eleven body areas as shown in Fig. 3.

Fig. 2

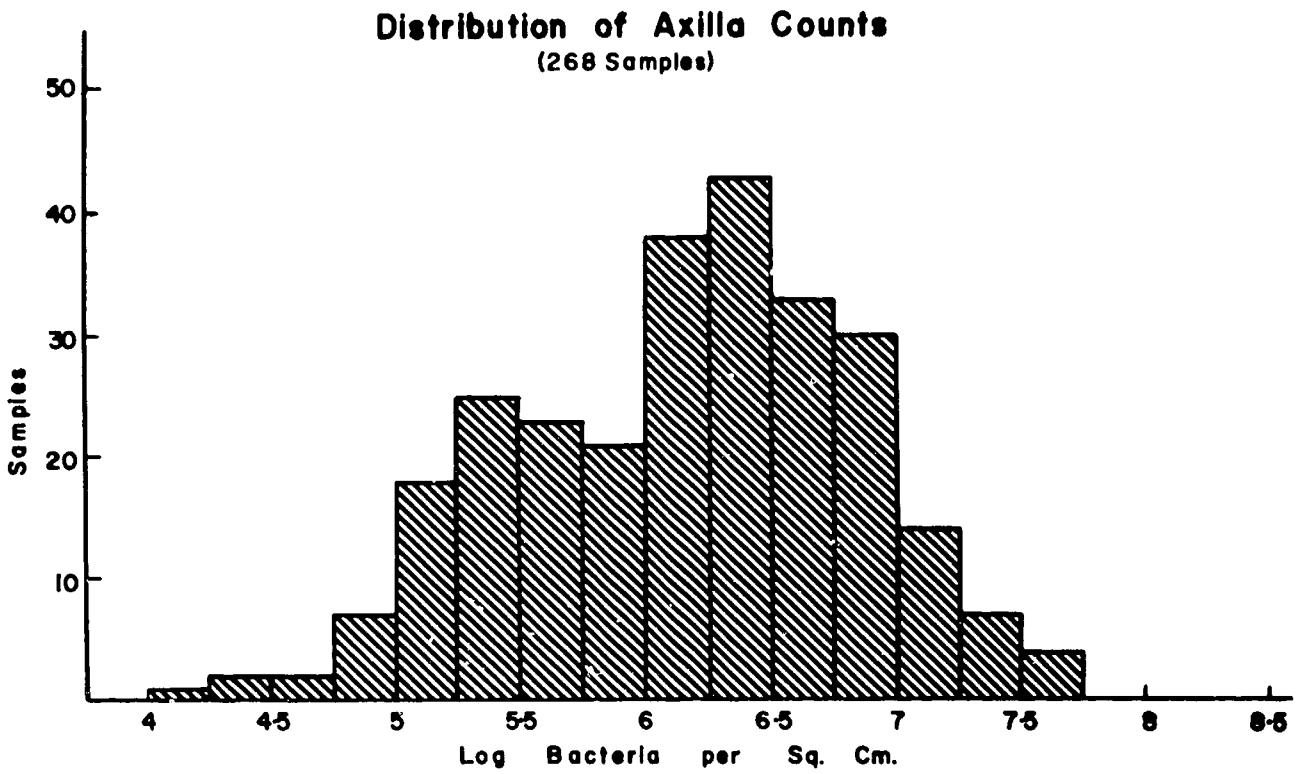


Fig. 3
Normal Flora - Bacteria per square centimeter

Site	# of Samples	Aerobic bacteria	Anaerobic bacteria
Axilla	239	1.55×10^6	336×10^3
Forearm	182	753	20
Forehead	59	44.5×10^3	2.143×10^6
Scalp	47	341×10^3	1.4×10^6
Cheek	12	123×10^3	13.6×10^6
Abdomen	19	1850	----
Back	51	261	----
Calf	12	451	----
Thigh	3	3750	----
Palm	20	1587	----
Back of hand	13	1326	----

The organisms comprising the flora in certain of these areas is shown in Fig. 4 which depicts the percentage of individuals from whom the particular organism was isolated.

Fig. 4
Percentage Incidence of Common Skin Bacteria on Representative Sites

	Forearm	Axilla	Scalp	Forehead
Number of Samples	83	57	53	55
S. epidermidis	98.8	93.0	100	94.3
S. aureus	3.6	3.5	0	1.8
S. rubens	6.0	10.5	0	1.8
M. luteus	91.6	21.1	36.4	28.3
Other cocci	36.1	47.4	88.7	49.1
Lipophilic Diphtheroids	75.9	96.5	47.5	77.4
Other aerobic diphtheroids	44.6	50.9	14.5	11.3
C. acnes and other anaerobic diphtheroids	33.3	73.3	72.0	92.5
Gram negative rods	22.9	31.6	19.4	13.2
Bacillus spp.	49.4	1.8	1.9	11.3
Yeasts	4.8	3.5	50.0	----

With regard to numbers, the findings agree with past appraisals;[9] wet areas support a large number of organisms as do regions of high sebaceous activity. The flora is more diverse in moist areas. There are vast regional differences. Individual variations are great. As a rule, the aerobic flora of the forehead, extremities and scalp is predominantly cocci of the Staphylococcus-Micrococcus complex. Lipophilic diphtheroids dominate the axilla. In contradistinction to previous concepts,[10] it is only on the head that the anaerobic corynebacterium exceeds the aerobic flora numerically.

It has become apparent that there are many more species of resident organisms than previously appreciated. These are not present on all skins but may be regularly isolated from certain individuals. When these minor resident organisms are taken into account, the differences among individuals become really quite striking; this, of course, holds true for the numbers of organisms.

These studies have so far been limited to adult males. It is eventually planned to characterize the flora in relation to age, sex, and race. We already know that negro skin supports a more numerous and perhaps a more diversified flora than white. This may reflect greater sweating. We have repeatedly had the opportunity to observe higher counts and unusual organisms on individuals working in moist, warm atmosphere such as cooks, launderers, etc. Our eventual objective is to understand the intrinsic and extrinsic factors which govern the bacterial ecology of the skin.

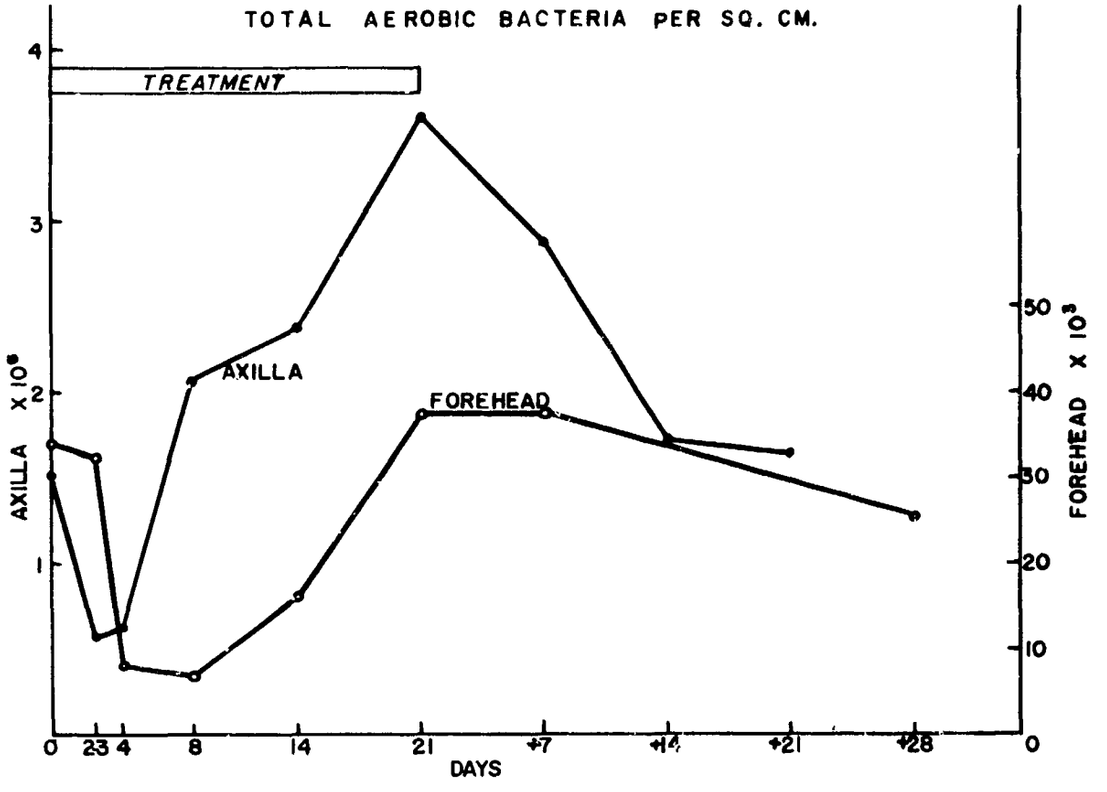
III. Systemic Antibiotics

The ecologic disturbances in the bacteriology of the nasopharynx and gastro-intestinal tract occasioned by the use of oral antibiotics have been vigorously

studied. The hazards of increasing the number of carriers of virulent, antibiotic-resistant organisms have been emphasized. Comparable studies have not been done for the skin. Almost nothing is known about the effects of long term antibiotic administration on the composition and numbers of the microflora.

We investigated the quantitative and qualitative effects of administering 600 mg of Demethylchortetracycline (Declomycin) for three weeks. The areas studied were the axilla, forehead, and back. Twenty-two sites were sampled for each area. Each sample was analyzed for total number and for the proportion of individual bacterial types.[11]

Fig. 5



A pattern of changes emerged which differed somewhat in the different areas. There was a rapid drop in total numbers in the first few days, more speedily in the axilla. (Fig. 5) However, after four to seven days, the numbers began to increase reaching the original level in the axilla by a week and on the forehead by three weeks. (Fig. 6) This rapid rebound was completely accounted for by the development of resistance by the original microflora. (Fig. 7) The composition of the flora was not altered; in the axilla however, the proportions of resident organisms were changed. (Fig. 8)

The appearance in the axillae of two individuals of large numbers of resistant S. aureus during the depression phase suggests that oral administration of antibiotics may entail epidemiologic hazards. Even though these virulent organisms disappeared with continued treatment, it is a matter of considerable importance to investigate this phenomenon

further, especially in those individuals who initially carry S. aureus in their nares or on the skin. A question which immediately arises is how quickly dermatitic skin which is so commonly colonized by S. aureus acquires an antibiotic resistant flora. It is already clear enough

Fig. 6
Population changes during Declomycin Treatment

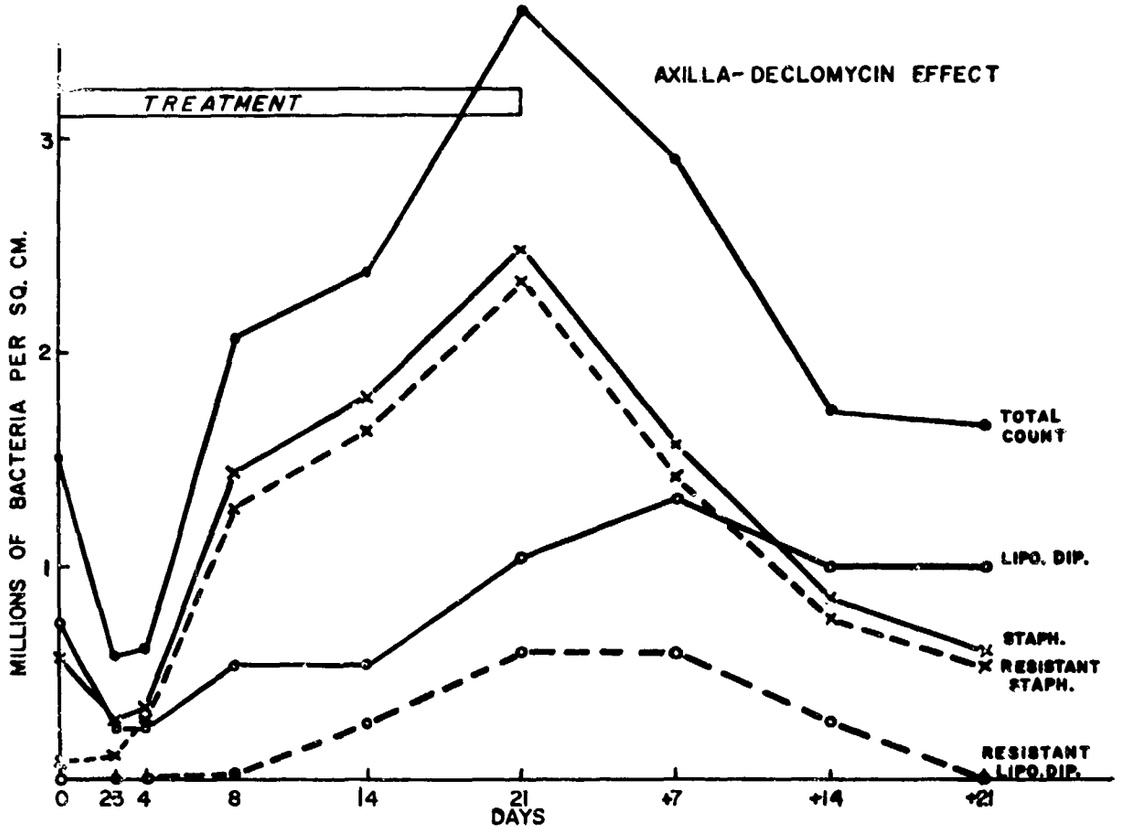
Day	Axilla			Forehead	
	% Cocci	% Lip. Dip.[1]	Other[2]	% Cocci	% Others[3]
0	38.0	48.7	13.3	91.0	9.0
2 + 3	48.5	45.9	5.6	93.7	6.3
4	54.8	39.9	5.3	91.9	8.1
Treatment					
7 + 9	69.1	26.3	4.6	85.1	14.9
14	74.9	23.0	2.1	91.5	8.5
21	69.0	28.9	2.1	94.4	5.6
+7	54.0	45.5	0.5	96.7	3.3
+14	48.7	49.7	1.6	90.4	9.6
Follow-up					
+21	37.0	59.5	3.5	92.1	7.9
+28	37.8	58.9	3.3	96.5	3.5

1. Lipophilic diphtheroids.
2. Mainly non-lipophilic diphtheroids and Gram-negative rods.
3. Mainly lipophilic diphtheroids and aerobic spore formers.

Fig. 7
Resistance Pattern
(Percent resistant of total number of each species)

Day	Axilla		Forehead	
	Staphylococci	lipophilic diphtheroids	Staphylococci	lipophilic diphtheroids
0	18.0	0	4.0	5.6
3	92.7	0.2	6.0	2.1
8	93.6	15.1	21.5	26.8
14	98.9	33.2	74.5	10.2
21	97.5	68.8	86.1	17.2
+7	94.6	59.4	94.7	30.4
+14	96.8	26.3	85.3	8.0
+21	96.0	-	77.3	-
+30	76.3	1.6	64.3	34.6

Fig. 8



that the skin should be more closely scrutinized when sick persons receive oral antibiotics especially in hospitals.

Preliminary study indicates that not all antibiotics behave in this fashion. A resistant flora, for example, does not seem to develop after the oral administration of penicillin. The likelihood of this occurrence seems to be dependent on whether the antibiotic reaches the cutaneous surface either via secretions, in sweat for instance, or as a result of being carried there by keratinizing epidermal cells. This has prompted us to study the distribution of antibiotics within the various skin strata, the dermis, epidermis and horny layer. Antibiotic assay shows that the tetracyclines and erythromycin are distributed in the epidermis and mainly reach the surface by the outward movement of keratinizing cells. On the other hand, penicillin is not detectable in the epidermis or horny layer. This agrees with the observation that penicillin does not appreciably alter the cutaneous microflora.

IV. Topical Antibacterial Agents

Whether justified or not, antibacterial agents are now widely incorporated in a great many products destined for application to the skin, viz. soaps, powders,

cosmetics, etc. Increasing efforts are being made to find antibacterial substances which are substantive to skin and which will have the effect of depressing the cutaneous flora incidental to daily hygienic practice, especially during bathing.

Fig. 9
Hexachlorophene - Powder versus Soap

	Subjects	Pretreatment counts (millions)	Post-treatment counts (millions)	% Reduction
Powder (1%)	20	6.354	1.365	78.5
Soap (1%)	80	5.549	0.62	88.9

We have compared the effectiveness of powders and soaps containing various antibacterial agents on a number of axillary organisms. The preparations were used once daily for ten days; the results are shown in Fig. 9 and 10.

Fig. 10
Comparisons between antibacterial soaps

	Subjects	Pretreatment counts	Post-treatment counts	% Reduction
Hexachlorophene (1%)	80	5.549	0.62	88.9
Safeguard Soap	70	7.214	0.136	98.1
Dial Soap	20	4.142	0.167	96.0
Ivory Soap	20	8.433	7.136	15.4
Camay	20	5.842	4.238	27.5

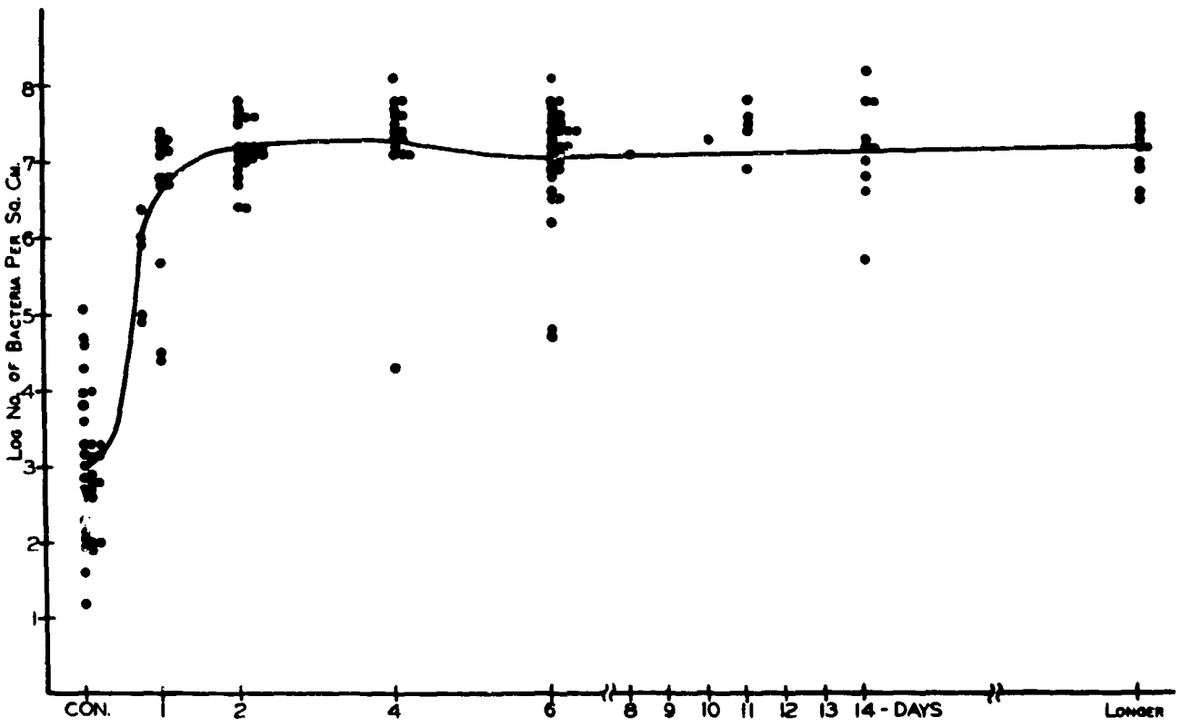
It will be seen that all of these preparations achieve a substantial reduction in bacterial numbers. Resistance has not been observed nor is there a change in the kinds of organisms present. Despite the high moisture content of the axilla, the use of agents selectively active against the predominant Gram positive microflora does not lead to replacement by Gram negatives. This occurrence is probably prevented because the Gram positives are not completely extinguished. Experience has shown that the survival of a small proportion of Gram positives is sufficient to hold the Gram negatives in check. This may not be true of the feet where Gram negatives are normally more numerous and may exclude other species. Despite the apparent safety record of antibacterial soaps, one should not dismiss the possibility that the effects may occasionally be harmful as a result of ecological shifts.

V. Growth of Bacteria Under Impermeable Plastic Dressings

The use of Saran Wrap is now widespread in clinical practice for the purpose of improving absorption of corticosteroid preparations. Occlusion with Saran Wrap is also

a very interesting technique to investigate the ecologic forces involved when a bacterial population is released from the restraints of dryness which normally prevail on the surface. We have shown on the forearm that the aerobic flora increases from less than 1,000 per sq. cm. to more than 10^7 in 48 hours. (Fig. 11) The component species do not increase at the same rate—the cocci respond first, to be succeeded by lipophilic diphtheroids and finally by non-lipophilic diphtheroids. (Fig. 12) If the occlusion continued for a week significant numbers of Gram negatives enter. Partial occlusion dampens all these effects. On the other hand, super-hydration by wet dressings leads to higher total counts and an earlier establishment of an augmented Gram negative flora.[12]

Fig. 11
Bacterial numbers on occluded forearm skin



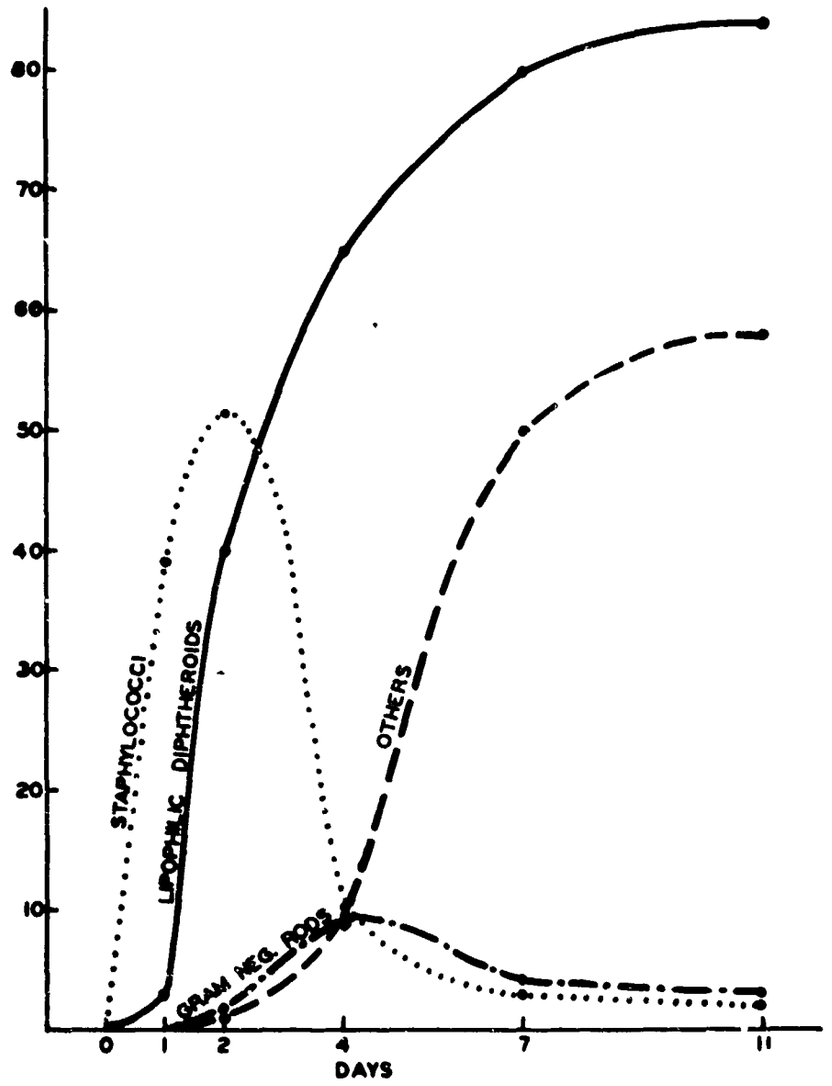
The occlusion technique provides an experimental system for testing the effectiveness of topical antibiotics. The criterion is the minimal effective level per sq. cm. that will prevent the increase in numbers. For example, the established usefulness of neomycin is confirmed by the finding that as little as $2u/cm^2$ is effective.

VI. Experimental Infections

Despite extraordinary efforts, past investigators have had little success in producing experimental S. aureus infections in human skin.[13,14] S. aureus rapidly disappears even when applied in huge quantities under occlusion. An experimental model which

is reproducible and quantifiable [illegible] be useful for evaluating topical medicaments under controlled conditions. Our preliminary results have been encouraging. The skin is first hydrated under Saran Wrap for 24-48 hours; the area is then lavaged copiously with 95% ethyl alcohol for one minute. This kills more than 90% of the organisms. Inoculating with S. aureus and covering with Saran Wrap enables infections to occur in a majority of instances. These are superficial and regress rapidly when the occlusion is removed. Only antibiotic sensitive strains are used. It is hoped to establish dose-effect relationships.

Fig. 12
Population changes on occluded forearm skin



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13. ABSTRACT

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It is unequivocally established that bacterial skin infections, primary or secondary, are a major source of disability, especially in tropical theatres. Moreover, a significant proportion of new arrivals experience sweat retention with all this entails in impairment of heat regulation and miliarial eruptions.

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